Tryptophan and kynurenines: continuing to court controversy.

Commentary on:
"The end of the road for the tryptophan depletion concept in pregnancy and infection."
By A. A-B. Badawy, A.M.A Namboodiri and J.R.Moffett

Trevor W Stone

Institute of Neuroscience and Psychology, College of Medical, Veterinary and Life Sciences, West Medical Building, University of Glasgow, Glasgow G12 8QQ, UK

Correspondence:-
Prof. T. W. Stone,
Institute of Neuroscience and Psychology,
University of Glasgow,
West Medical Building,
Glasgow G12 8QQ, UK
Tel:- (0044)-(0)141-330-4481;
e-mail:- Trevor.Stone@glasgow.ac.uk; trevorwstone@btinternet.com

The "essential" amino acid tryptophan has a fascinating history. Even its discovery by Gowland Hopkins at the beginning of the twentieth century, in accidental observations reputedly stemming from the presence of glyoxylic acid contamination in bottles of acetic acid, was quite unusual. The description "essential" is not strictly appropriate since large numbers of bacterial cells within the gut synthesise tryptophan which can enter the host circulation.

In the decades following the discovery of tryptophan it became clear that this amino acid was the primary mammalian origin for the synthesis of nicotinic acid and nicotinamide (forms of niacin, vitamin B3) and the co-factor nicotinamide adenine dinucleotide (NAD), a biochemically versatile compound essential for the functioning of many mammalian enzymes. The pathway from tryptophan to nicotinamide became known as the kynurenine pathway, named after the first stable compound generated by the oxidation of tryptophan. Kynurenine itself, however, appeared to be biologically inactive and, as a result, the pathway between tryptophan and nicotinamide held little interest. This attitude persisted until two new chapters were opened in the early 1980s. One of these, most directly relevant to the article by Badawy et al. [1] was the observation that interferon-ɣ, produced by mammalian cells when exposed to infection or inflammatory conditions, powerfully activated the first enzyme of the extra-hepatic kynurenine pathway, indoleamine-2,3-dioxygenase (IDO). This activation was associated with a suppression of the proliferation of infecting organisms and, since that suppression could be prevented by raising the tryptophan levels (in cell cultures), the mechanism of suppression was simplistically attributed to the reduction in tryptophan preventing protein synthesis and cell division [2-4]. However, this "tryptophan depletion" concept was challenged from its outset by the opposing proposal that the further metabolites of tryptophan generated along the kynurenine pathway might be involved in the anti-infective activity via some form of toxic action on the invading cells, rather than a simple reduction in tryptophan levels.

It was to take another five years before the same principle of tryptophan depletion was invoked to explain one of the most important phenomena in biology. This was the finding that kynurenine pathway activity was required for successful implantation, growth and development of an embryo. Inhibiting the first enzyme of the pathway, IDO, in pregnant animals (using 1-methyl-tryptophan) led to spontaneous abortion and the loss of most embryos [5]. It was suggested that this phenomenon arose from IDO activity in the foetus or placenta which normally maintained local tryptophan concentrations at a low level by oxidising it to kynurenine. That subnormal level of tryptophan was thought to inhibit the proliferation and activity of maternal immune system T cells which form part of the surveillance system that seeks out and destroys foreign cells. By blocking IDO, therefore, this T cell suppression could not occur and the T cells could then attack and kill the embryonic cells as if they were a foreign (allogeneic) organism.

Fascinating though this idea was, it was soon tempered by the increasing acceptance that kynurenine metabolites had a range of actions on cells of the immune system, including T cells [6]. Thus, for more than 20

years there has been an unease in the tryptophan community, especially its kynurenine pathway branch, with a lack of clarity and confidence in the mechanistic consequences of IDO activation, an unease compounded by the fact that if the kynurenine pathway is a fundamental requirement for successful reproduction and for anti-infective immune defences, it is one of the most important pathways in the mammalian body and one which should be better understood.

It is in this context that the review by Badawy et al. [1] should be viewed. The authors have brought together the arguments for and against the tryptophan depletion concept, criticising the former by a series of reasoned arguments and reminders that tryptophan regulation is an extremely complex phenomenon in which all the components must be considered. For example, the high affinity but low capacity enzyme IDO does not control overall tryptophan metabolism. Firstly, there are at least two isoforms, IDO-1 and IDO-2, which are active in different tissues under different conditions. Secondly and more importantly, the largely hepatic enzyme tryptophan-2,3-dioxygenase (TDO, also existing in two isoforms) has a much higher capacity and, under normal, resting conditions, will be the prime regulator of systemic tryptophan levels, likely to mask any effects of IDO in placental, foetal or immune system cells.

Another serious argument is that systemic tryptophan levels *in vivo* could not be lowered enough to starve attacking cells without compromising the health of mother or foetus. This might be partially countered by noting that local changes in tryptophan concentrations could be generated by locally activated IDO, without the need to change circulating levels that affect the whole organism. Activity of IDO is normally low until it is activated by interferons and cytokines as part of the inflammatory and anti-infective activity of the immune system, when it might be able to control local tryptophan concentrations in the immediate vicinity of susceptible cells. Indeed, one might speculate that the ability to regulate systemic tryptophan levels via hepatic TDO in the face of varying food intake or periods of starvation, with IDO serving as a regulator of local tryptophan concentrations depending on inflammatory activation, could have contributed to the evolution of these two parallel, but distinct enzymes performing similar biochemical functions. Although induction of IDO is obvious with infection, however, a clearer view of how it is induced and regulated in the foetus and placenta would be useful. Oestrogens can reduce IDO activity in the brain as well as inhibiting TDO in the liver, but there is existing evidence that oestrogens activate or up-regulate IDO in the placenta and progestogens down-regulate the enzyme. Regional differences such as these would be highly relevant to understanding the overall functional role of kynurenines in pregnancy and the overall dynamics of the kynurenine pathway in pregnancy merit more detailed analysis at the cellular and biochemical levels.

But if the depletion hypothesis is no longer tenable, is the concept that increased formation of kynurenine metabolites - the "tryptophan utilisation" hypothesis championed by Badawy et al.[1] – a viable alternative explanation for the local control of immune system activity? A major difficulty often raised with this hypothesis is that raised tryptophan levels oppose the cell suppressant effect of IDO activation. If IDO activity works by reducing local tryptophan concentrations, this makes sense viewed as a simple restoration of normal tryptophan availability. However, if IDO activation suppresses cell viability by increasing the formation of kynurenine metabolites, then that activity should be potentiated by supplying higher amounts of tryptophan. Badawy et al. [1] try to resolve this issue by reminding us that excessive amounts of tryptophan, over around 200μM, can inhibit IDO. Thus, high local concentrations of tryptophan should not be expected simply to counteract the effects of local depletion. Such an explanation would certainly help understanding of these inter-relationships, but it is one which also requires more intensive analysis and understanding of the kinetic interactions between all the enzymes and metabolites in the kynurenine pathway

It is valuable to be reminded by Badawy et al. [1] of problems in the experimental design or tools used in previous work on tryptophan biology. One problem is undoubtedly the failure to consider the roles of bound and free tryptophan. Most studies measure total plasma levels, but these can vary independently of local concentrations. Indeed bound tryptophan will act as a buffer reservoir in most situations, combatting any tendency for tryptophan levels to fall. Total plasma levels are known to fall during pregnancy as a result of increased uptake into cells which is probably necessary to maintain healthy cells in the mother in parallel with intense new growth activity in the foetus. Yet *free* tryptophan levels are increased in pregnancy, a phenomenon which would not fit with the depletion hypothesis of T cell suppression.

Equally, in experimental conditions where more direct interference with enzyme activity is possible, tools that are sufficiently selective have not been available until recently. One of the key compounds used to block IDO activity and thus inhibit the kynurenine pathway was 1-methyl-tryptophan, but it is now recognised that this compound also inhibits tryptophan transport and up-regulates IDO expression. The latter, of course, might be secondary to the former, but interpretation remains difficult and more detailed work is required. There remain questions about the interactions between kynurenine metabolites that might complicate interference with tryptophan availability. For example it is clear that inflammation is associated with a change in the balance between 3-hydroxy-anthranilic acid and anthranilic acid concentrations [7]. The ratio between these compounds seems to correlate with inflammatory activity and anthranilic acid is the structural basis of many recognised and

experimental anti-inflammatory drugs. How does this fit with the actions of these compounds on the immune system and their relative amounts in the face of changes in IDO activity and tryptophan availability?

Why is all this important? Three reasons. Firstly, the same self-protection generated by IDO activity in the foetus and placenta also applies to many cancer cells. IDO expression is high in these cells, resulting in T cell suppression and cancer cell escape from detection and destruction. Producing inhibitors of IDO has become big business, with most pharmaceutical companies generating them to block IDO in cancer cells, thus reducing their resistance to immune attack and increasing their susceptibility to traditional chemotherapeutic agents. Given the doses and long periods for which anti-cancer drugs are often required, it is important to understand fully how these compounds work and what other long-term effects might be produced or influenced by kynurenine metabolites.

Secondly, there is a growing interest in the overlap between kynurenines in the immune system and their effects on the nervous system. The other new chapter on the kynurenine pathway in the 1980s actually began two years before Pfefferkorn's paper [4], with the discovery that one component of the pathway – quinolinic acid, the precursor of nicotinic acid – excited neurons in the cerebral cortex by acting on glutamate receptors sensitive to N-methyl-D-aspartate (NMDA) [8] and were becoming a focus of attention as they possessed unique properties of fundamental importance in a range of cerebral phenomena. These included synaptic plasticity (implying a role in learning and memory processes and all the cognitive functions based on these), cyclical depolarising shifts (which implied a possible role in abnormal excitability states such as epilepsy), and the promotion of calcium influx (which implied relevance for excitotoxicity and neurodegenerative disorders such as Alzheimer's disease, Huntington's disease and strokes) [8]. Even more intriguing was the subsequent discovery that another metabolite of kynurenine – kynurenic acid – was an antagonist at these same NMDA-sensitive receptors, leading to the concept that the balance of concentrations of quinolinic acid and kynurenic acid could be of fundamental importance in normal and abnormal brain function [8]. By regulating both immune system activity and the excitability and synaptic function in the nervous system, tryptophan has therefore become a key player in the integration of these systems. Changes in the immune system will alter kynurenine pathway activity and, secondarily, brain function. Conversely, neuronal and the associated glial cell activity in the brain, will alter kynurenine pathway activity which could affect immune cell function peripherally. Is this why we have difficulty thinking and focussing when we have an infection or significant inflammation peripherally? Is it why the activity of our immune system – and thus our sensitivity to infections and cancer – depends on our state of mental activity, mood and outlook? Is this how psychological treatments, faith healing and placebos work their magic? The kynurenine pathway may be the key to a new era of understanding and the design of integrated, holistic approaches to health and medicine.

Thirdly, raising these many problems and discrepant interpretations should encourage a more detailed and systematic analysis of the inter-relationships between the components of tryptophan metabolism. An outstanding example of this is the need integrate the finding that IDO has non-enzymic functions in the regulation of immune dendritic cells [9]. This, and perhaps other unidentified features of tryptophan metabolism serve to emphasise how multi-faceted and fundamental this pathway is in mammalian biology.

Lastly, in writing their review, Badawy and colleagues [1] also correct some accumulated, historical errors of citation. Sometimes claims to the importance of earlier work reveal incidental observations that would have had no meaning in themselves until they were placed into an explanatory, functional, biologically meaningful context by later work, so that the earlier citations are not appropriate. In the case of IDO, however, the first indication of its induction by infection [2,3] has long been overlooked and, as proposed by Badawy et al. [1], ought to receive the credit due.

REFERENCES

[1] Badawy, A.A-B., Namboodiri, M.A., Moffett, J.R. (2016) The end of the road for the tryptophan depletion concept in pregnancy and infection. Clin. Sci.

[2] Yoshida, R. & Hayaishi, 0. (1978) Induction Of Pulmonary Indoleamine 2,3-Dioxygenase By Intra-Peritoneal Injection Of Bacterial Lipopolysaccharide. Proc. Nat. Acad. Sci. USA 75, 3998-4000.

[3] Yoshida, R., Urade, Y., Tokuda, M. & Hayaishi, 0. (1979) Induction Of Indoleamine 2,3-Dioxygenase In Mouse Lung During Virus-Infection. Proc. Nat. Acad. Sci. USA 76, 4084-4086.

[4] Pfefferkorn, E. R., Eckel, M. and Rebhun, S. (1986) Interferon-gamma suppresses the growth of Toxoplasma gondii in human fibroblasts through starvation for tryptophan. Mol. Biochem. Parasitol. **20**, 215-224.

[5] Munn, D. H., Zhou, M., Attwood, J. T., Bondarev, I., Conway, S. J., Marshall, B., Brown, C. and Mellor, A. L. (1998) Prevention of allogeneic fetal rejection by tryptophan catabolism. Science **281**, 1191-1193.

[6] Fallarino, F., Grohmann, U., Vaccia, C., Bianchi, R., Orabona, C., Spreca, A., Fioretti, M.C., Puccetti, P. 2002. T cell apoptosis by tryptophan catabolism. Cell Death Differentiation 9, 1069-1077.

[7] Darlington LG, Forrest, C.M., Mackay, GM, Stoy, N, Smith, RA, Smith AJ, Stone TW (2010). On the biological significance of the 3-hydroxyanthranilic acid:anthranilic acid ratio. Internat. J. Tryptophan Res. 3, 51-59.

[8] Stone, T.W., Darlington, L.G. (2002). Endogenous kynurenines as targets for drug discovery and development. Nature Revs. Drug Disc. 1, 609-622.
[9] Pallotta, M.T., Orabona, C., Volpi, C., Vacca, C., Belladonna, M.L., Bianchi, R., Servillo, G., Brunacci, C., Calvitti, M., Bicciato, S., Mazza, E.M.C., Boon, L., Grassi, F., Fioretti, M.C., Fallarino, F., Puccetti, P., Grohmann, U. (2011) Indoleamine 2,3-dioxygenase is a signaling protein in long-term tolerance by dendritic cells Nature Immunol. 12, 870-878.